PATENT

IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re the application of:

Tero SOUKKA et al.

Serial Number: 10/551,690 Group Art Unit: 1641

Filed: February 17, 2006 Examiner: Yu, Melanie J.

For: NANOPARTICLE FOR BIOAFFINITY ASSAYS

DECLARATION PURSUANT TO 37 C.F.R. § 1.132

Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

Sir:

I, Urpo LAMMINMÄKI, hereby declare as follows:

1. I am one of the co-inventors of the nanoparticle disclosed and claimed in the above-captioned application.

2. The claimed nanoparticle is a recombinant apoferritin particle (or a recombinant Dpr protein particle or recombinant Dps protein particle) in which at least first binding moieties are genetically fused to protein and/or peptide subunits which form a shell having an inner surface and an outer surface.

3. The terms "genetic fusion" and "genetically fused" are widely used in the scientific literature when referring to a gene construct encoding a fusion protein. See, for example, Massa et al., "Antitumor Activity of DNA Vaccines Based on the Human Papillomavirus-16 E7 Protein **Genetically Fused** to a Plant Virus Coat Protein," 19 Hum. Gene Ther. 354 (2008)(emphasis added), Schulte, "Use of Albumin Fusion Technology to Prolong the Half-life of Recombinant Factor VIIa," 122 Thromb Res. Suppl 4:S14 (2008)("One such approach uses albumin fusion technology in which human albumin is **genetically fused** to the C-terminus of rFVIIa via a flexible glycine serine linker," Abstract, emphasis added), and Scerbo et al., "Protective Effect of a Synapsin Peptide **Genetically Fused** to the B Subunit of *Escherichia coli* Heat-labile Enterotoxin in Rat Autoimmune Encephalomyelitis," J Neurosci Res. (2009) (Epub ahead of print)(emphasis supplied).

4. A "fusion protein" is the product of two proteins or peptides which are linked to each other at the genetic level such that the chemical structure of the fusion protein is unambiguously determined by the genetic information of the two proteins or peptides. Genetic fusion results in the production

of a single polypeptide. See Subramanian et al., "Albinterferon α-2b: A Genetic Fusion Protein for the Treatment of Chronic Hepatitis C," 25 Nat. Biotechnol. 1411 (2007), where the two proteins or peptides forming the fusion protein are always present and where they are conjugated to each another site-specifically, i.e., there is a single fusion site in the fusion protein. ("During the fermentation process, the gene is transcribed and translated as a single polypeptide, which is then secreted into the fermentation broth." Id. at 1413, left col., lines 19-20, emphasis added.)

5. In the present invention, a first binding moiety and a ferritin subunit are genetically fused to one another and therefore there is a single fusion site in the resulting fusion protein. The resulting fusion protein has a defined, uniform, covalent structure and comprises, in this case, one binding moiety and one ferritin subunit. Due to the intrinric self-assembly capability of the ferritin moiety, the fusion proteins will assemble into uniform nanoparticles comprising 24 identical fusion proteins - i.e., all ferritin subunits which have been genetically fused to a first binding moiety are identical to one

another, such that the binding moieties are each located at the same place in the subunit's polypeptide chain.

6. Ferritin nanoparticles are conventionally prepared by chemical conjugation, in which conjugation takes place at a chemical rather than the genetic level, i.e., at the level of existing proteins. There are numerous examples in the scientific literature showing that when individual proteins are modified using chemical conjugation-based approaches the resulting product is heterogeneous, not uniform. See Kitamura et al., "Chemical engineering of the monoclonal antibody A7 by polyethylene glycol for targeting cancer chemotherapy," 51 Cancer Res. 4310 (1991); and Clark et al., "Long-acting growth hormones produced by conjugation with polyethylene glycol," 271 J Biol Chem. 21969 (1996). The reason behind the product heterogeneity is that proteins generally contain several identical or chemically similar reactive groups, any of which can become targeted by the chemical conjugation reaction. Consequently, chemical conjugation can occur in different positions in protein molecules. The problem of product heterogeneity is increased if both molecules to be conjugated

contain multiple chemically reactive groups, which can be the case if two proteins or peptides are conjugated.

7. The problem of heterogeneous reaction products produced by chemical reaction based conjugation is particularly severe in the case of nanoparticles composed of multiple identical protein subunits (such as ferritin), because the number of chemically reactive groups present in one subunit are multiplied by the number of subunits in the particle. For example, the ferritin nanoparticle (comprising 24 subunits) would contain a minimum of 24 reactive groups. Furthermore, non-specific side reactions which commonly occur during chemical conjugation can further increase the level of heterogeneity, especially if an excess of conjugation reagent is employed.

8. The advantages of genetic fusion-based production of ferritin nanoparticles include production of homogeneous nanoparticles having constant orientation/conformation of binding moieties without using complex chemistry. See Choi et al., "Glutamate Decarboxylase-derived IDDM Autoantigens Displayed on Self-Assembled Protein Nanoparticles," 327 Biochem Biophys Res Commun. 604 (2005) at page 606, left col., lines 12-

22, and Lee et al., "A Novel Approach to Ultrasensitive Diagnosis Using Supramolecular Protein Nanoparticles," 21 FASEB J. 1324 (2007) at page 1324, right col., lines 22-35.

9. I understand claims 26, 27, 29 and 36-38 of this application have been rejected as anticipated by U.S. Patent No. 4,959,306 to Kameda et al.

10. Example 1 of Kameda et al. uses chemical conjugation to bind a linker (sulfo-SMCC) to an apoferritin nanoparticle in a first step, followed by chemical conjugation of an activated Fab fragment to the apoferritin-linker complex in a second step. The resulting nanoparticles were determined to contain five binding moieties (Fab' fragments) per apoferritin nanoparticle. As ferritin contains 24 subunits this procedure results in particles having a non-equimolar ratio of binding subunits and ferritin subunits. On a molecular level individual nanoparticles are different, because it is not possible to determine or control which ferritin subunits are targeted in the conjugation reaction. In other words, the Fabs are randomly distributed in the apoferritin nanoparticles and the conjugation procedure

results in a mixture comprising an extremely high number of structurally different apoferritin nanoparticles.

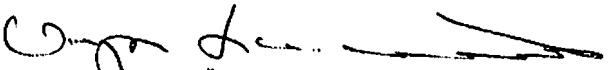
11. In my opinion, the claimed nanoparticle is structurally different from the nanoparticle disclosed in Kameda et al. because, in the claimed nanoparticle, all the subunits of the ferritin in which a first binding moiety has been genetically fused are identical to one another, such that the binding moieties are each located at the same place in the polypeptide chain. This uniform structure is not present in the Kameda et al. nanoparticle, which is produced by chemical conjugation.

12. In my opinion, a genetic fusion-based approach is the only practical method which can produce uniformly derivatized nanoparticles in the case of ferritin nanoparticles which comprise multiple identical subunits. Furthermore, the genetic fusion-based approach allows simpler production of ferritin nanopartices endowed with binding moieties than a chemical conjugation-based approach. Ferritin nanoparticles generated by a genetic fusion-based approach already contain the binding moieties when produced by the cell (or when self-assembled from the ferritin fusion protein produced by the cell). In contrast,

if a chemical reaction-based conjugation is used the nanoparticles produced by the cells (or self-assembled from the ferritin subunits produced by the cell) need to be subjected to additional chemical reaction(s) in order to introduce the binding moieties.

13. I declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and that further these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code, and that such willful false statements may jeopardize the validity of the application or any patent resulting therefrom.

Signed this 3rd day of April, 2009.

Signed: 

Urpo LAMMINMÄKI